ASSOCIATION OF FSHR GENE POLYMORPHISM WITH POLYCYSTIC OVARY SYNDROME IN IRAQI WOMEN

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ABSTRACT

This study aims to detect the relationship between FSHR gene polymorphism and Polycystic Ovary Syndrome in Iraqi women using polymerase chain reaction –restriction fragment length polymorphism (PCR-RFLP). Fifty blood samples from Iraqi women with polycystic ovary syndrome (age ranged 16-45 years) and twenty five blood samples from healthy women were collected from Kamal Al-Samarrai Hospital/ Baghdad, during the period from October 2014 to May 2015. DNA extraction using Bionear kit and the fragments of FSHR gene that contains the SNP rs6165 were amplified by PCR and then products were analyzed by RFLP using restriction enzyme AhdI. The new SNP rs6165 on FSHR shows no significant differences between patients and control group, which indicate the highly conservative sequence in Iraqi women. This is the first study to examine the association of FSHR gene polymorphism in an Iraqi ethnic to the best of the research knowledge.

KEYWORD: Polycystic Ovary Syndrome - FSHR gene polymorphism - PCR-RFLP.

INTRODUCTION

Polycystic ovarian syndrome is one of the most common endocrine disorders affecting women in the reproductive age, it is a genetic disorder that can be inherited from either parents. It is usually diagnosed during the early reproductive years. It is a life-long lasting condition with substantial variation of symptomatology regarding reproductive, metabolic and cardiovascular health throughout a woman's life. As many as 5 million women in the United States may be affected. It can occur in girls as young as 18 years old.\textsuperscript{[1]}
The features of (PCOS) are The menstrual irregularity, infertility, Hyperandrogenism, an inappropriate gonadotrophin secretion is associated with the classic form of PCOS, Obesity. There are different candidate genes as a cause of PCOS. Different studies have indicated a genetic susceptibility to PCOS; it was shown that polycystic ovaries and hyperandrogenemia are present in families of affected women. Therefore 50% genetic analyses of candidate genes have been performed. Both linkage and association studies have suggested that PCOS can be explained by the interaction of several genes with environmental factors. FSHR genes may be an important candidate gene for PCOS. Indeed, a previous genome-wide association study in Chinese women with PCOS identified a region on chromosome 2p16.3 that encoded the FSH receptor (FSHR) genes as a reproducible PCOS susceptibility locus. A few genetic studies have examined the association between FSHR gene polymorphisms and PCOS; FSHR gene is comprises of 10 exons. Most studies focused on two SNPs Thr307Ala (rs6165 and rs 6166).

Subjects and methods

This study includes fifty patients women with polycystic ovary syndrome with age range 16-45 years and Twenty five controls. The patients were selected from high institute for infertility diagnosis and assisted reproductive technologies, Al-Nahrain University. The patients with PCOS were diagnosed based on the presence of two out of three criteria of Rotterdam European Society for Human Reproduction and Embryology (ESHRE) including oligo- and/or unovulation, and clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries. DNA extraction was done using Bionear kit, The fragments of FSHR gene that contains the SNP rs6165 were amplified by PCR using specific primer supplied by Alpha DNA Company as a lyophilized product of different picomols concentrations. The master mix of PCR 12.5 µl was mixed with 3 µl of DNA and 1 µl from each primer forward and reverse and 7.5 µl of free nuclease water, the PCR program was carried out according to the instructions of the company to get 25 µl final volume (Table1).

Table 1 PCR Program

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>No. of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>94</td>
<td>5 min.</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94</td>
<td>25 sec.</td>
<td>35</td>
</tr>
<tr>
<td>Annealing</td>
<td>55</td>
<td>40 sec.</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
<td>30 sec.</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72</td>
<td>5 min.</td>
<td>1</td>
</tr>
</tbody>
</table>
RFLP analysis was done as follow Ten μl of amplified products was mixed with 4Units AhdI enzyme, 2 μl of enzyme buffer and 7.6 free nucleases deionized distilled water, then it was incubated for 3 hours in 25°C.[6] The enzyme digestion mixture was loaded in the well with 2% agarose gel stained with ethidium bromide at final concentration of 0.5μg/ml the gel was electrophoresed, at 5 Volt/cm for 1 hour, in 1 X TBE buffer. After that it was visualized under UV light using ultraviolet transillumenater. A 100-1000 DNA ladder was used, and the gel was photographed by a digital camera.

Statistical Analysis
The Statistical Analysis System- SAS (2012) was used to detect the effect of different factors in the studied of parameters. The least significant difference –LSD test was used for significantly comparing the means in this study.

RESULTS AND DISCUSSION
The fragments of FSHR gene that contains the SNP rs6165 was amplified the results of PCR analysis showed the size of the fragment was 577bp. To investigate the frequency of genotypes in FSHR, RFLP analysis was used as a tool. Restriction fragment length polymorphism (RFLP method) is a technique that exploits variations in homologous DNA sequences. It refers to the difference between samples of homologous DNA molecules from different locations of restriction enzyme sites.

So the PCR product (577 bp) was digested with AhdI restriction enzyme. The presence of two fragments (174 bp and 403 bp)(Fig 1) indicates normal homozygot (AA),while the presence of polymorphism AG heterozygous yielded 577, 403 and 174 bp fragment (Figure - 2). According to that 44 (88%) of PCOS patient were homozygous (AG) and the other 6 (12%) were heterozygous.

Table (2) Distribution of sample study according to the type of mutation in patients

<table>
<thead>
<tr>
<th>Type of mutation</th>
<th>No.</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo (AA)</td>
<td>44</td>
<td>88.00</td>
</tr>
<tr>
<td>Hetero (AG)</td>
<td>6</td>
<td>12.00</td>
</tr>
<tr>
<td>Chi-square value</td>
<td>----</td>
<td>13.826 **</td>
</tr>
</tbody>
</table>

** (P<0.01).
Figure (1): Restriction fragment length polymorphism (RFLP) analysis of the *AhdI* digest of the PCR product that contains the FSHR gene separated on a 2 % agarose gel. M: DNA ladder (100-1000) bp and Lanes (1,2,3,4 and 5) homozygous normal (A\A) , C: control.

Figure (2): Restriction fragment length polymorphism (RFLP) analysis of the *AhdI* digest of the PCR product that contains the *FSHR* gene separated on a 2 % agarose gel. Lane M DNA ladder 100-1000 bp; Lanes 1,2: un cut DNA, Lanes 3,4 and 5 Heterozygous mutants (A\G), C: control.

These results indicated that heterozygous mutations in the *FSHR* gene could cause PCOS. Diverse inactivating mutations and polymorphisms have been described in *FSHR* gene in women with ovarian dysfunction.\[^{6}\]\ Impairing follicle-stimulating hormone (FSH) signaling *in vivo*: targeted disruption of the *FSHR* leads to aberrant gametogenesis and hormonal imbalance. Follicle stimulating hormone receptor (*FSHR*) is located both in Sertoli cells of the testis and granulose cells of the ovaries.\[^{7}\]\ The mutations in *FSHR* can lead to arrest of follicle development at several phases of growth.\[^{8}\]\

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\[^{6}\] Impairing follicle-stimulating hormone (FSH) signaling

\[^{7}\] Follicle stimulating hormone receptor (*FSHR*) is located both in Sertoli cells of the testis and granulose cells of the ovaries.

\[^{8}\] The mutations in *FSHR* can lead to arrest of follicle development at several phases of growth.
There are a number of genetic variants in the FSHR that have an effect on the phenotype. These effects include variable development of secondary sex characteristics, primary amenorrhea, hypoplastic ovary and high serum levels of FSH. Prevalent polymorphisms of FSHR are found within either alanine (Ala) or threonine (Thr) and position 680 is occupied by either serine (Ser) or asparagine (Asn) in the intracellular domain of the protein. However, some research groups have reported the occurrence of the same polymorphisms located in different amino acid positions resulting in different allele genotyping. Other research groups have reported that the substituted nucleotide is different for the same polymorphism, and consequently an amino acid is differentially changed. For example, Ala307Thr is mentioned as Thr307Ala. Furthermore, polymorphisms at 307 and 680 codon of FSHR have shown diverse phenotypes among different ethnic groups and diseases. The important previous genome-wide association study on Chinese women with PCOS identified a region on chromosome 2p16.3 that encodes the FSHR gene as a reproducible susceptible locus for PCOS.

The new SNP rs6165 on FSHR shows no significant differences between patients and control group, which indicate the highly conservative sequence in Iraqi women.

This is the first study to examine the association of FSHR gene polymorphism in an Iraqi ethnic to the best of the research knowledge.

REFERENCES


